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| EXAMINER | |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/523,953 | ZHOU, GUOHUA | |
| | Examiner | Art Unit | |
| | Molly E. Baughman | 1637 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-10* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9* is/are rejected.

7)☒ Claim(s) *8 and 10* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *29 September 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/8/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Claim Objections*

1. Claim 8 is objected to because of the following informalities: "dideoxynecleoide" is misspelled and should read, "dideoxynucleotide." Appropriate correction is required.

2. Claim 10 is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternative only. See MPEP § 608.01(n). Accordingly, the claim has not been further treated on the merits.

### *Claim Rejections - 35 USC § 112*

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claims 1-9 are confusing because it cannot be determined what is encompassed by the term, "characterized in that" in claim 1. The scope of the phrase is unclear, and it is suggested to use conventional U.S. claim language, such as "comprising," or, "consisting of."

a. Claim 1 is confusing because it cannot be determined what is encompassed by "a base type and a signal intensity in a sequencing profile representing a gene source and relative expression level, respectively," in the step (c). While the breath of the phrase is understood, the phrase as written is

sequence, used to represent the particular gene source it's ligating to. Clarification is required.

f. Claim 6 is confusing because, as mentioned above, it appears the three methods of claim 4 should be in the alternative, however, the claim recites "selective adaptors and anchored primers," which are separately included in the three methods, and should therefore be referred to in the alternative. As such, it is also confusing which anchored primers the claim is referring to since the second method and the third method both use anchored primers. Correction is required.

g. Claim 6 recites the limitation "the part used for identifying gene sources in selective adaptors" in claim 4. There is insufficient antecedent basis for this limitation in the claim.

***Claim Rejections - 35 USC § 103***

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1-4, and 6-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uematsu et al. (US 6,225,064) in view of Kato (US 6,090,556) and Kaufman et al. (US 6,383,754) as evidenced by Ronaghi et al., "A Sequencing Method Based on Real-Time pyrophosphate," Science, 1998, Vol.281, pp.363 and 365 (of record).

grammatically confusing with the step as a whole and renders the step indefinite. Clarification is required.

b. Claim 3 is confusing because it is unclear how the claim further limits claim 1. Claim 3 describes the source-specific primers, however, the source-specific primers of claim 1 are limited to such a description, as defined by the specification. It also makes it unclear whether the source-specific primers of claim 1 could also encompass other definitions. Clarification is required.

c. Claim 4-6 are confusing because claim 4 recites a first, second and third method, and it is unclear how the suitable method of claim 1 could encompass all three methods since the three methods, as stated in claim 4, are not recited in the alternative. For purposes of examination, the claim will be interpreted as the method of claim 1, wherein the suitable method is any one of the three methods. Clarification is required.

d. Claims 4-6 are confusing because claim 1 is drawn to "...mixing the labeled mRNA fragments...," however, the method of claim 4 is drawn to suitable methods of labeling mRNA, which result in fragments which are cDNA, not mRNA. Clarification is required.

e. Claim 5 is confusing because it cannot be determined what is encompassed by "a sequence specific to gene sources." It unclear whether this sequence is a sequence specific to an actual target nucleic acid (i.e. the gene source), or an arbitrary sequence, used to represent the particular gene source it's ligating to. For purposes of examination, it will be interpreted as an arbitrary

Regarding claims 1-4, and 6-7, Uematsu teaches a method wherein the method comprises (a) labeling mRNA from different sources with a suitable method, and mixing the labeled mRNA fragments equally to obtain a template for polymerase chain reaction (PCR); (b) performing a polymerase chain reaction using source-specific primers and a gene-specific primer; and (c) detecting a sequence of amplified DNA fragments with bioluminescence analysis, a base type and a signal intensity in a sequencing profile representing a gene source and a relative expression level, respectively. The suitable method for labeling mRNA includes (the first method): performing a reverse transcription-polymerase chain reaction (RT-PCR) to obtain complementary DNA (cDNA) fragments of a given gene in each source; digesting cDNA into fragments with a suitable length using a restriction endonuclease; and ligating each of the digested cDNA fragments with a selective adapter, a different adapter corresponding to mRNA from a different source. Specifically, Uematsu et al. teaches a method of comparing the difference in expression profiles between plural samples wherein RNAs are extracted from plural samples from different sources (kidney and liver derived, see Example 1 [i.e. different organs of an individual, claim 2]), separately reverse transcribed, digested, ligated with different oligonucleotides (i.e. labeling the mRNA), mixed equally, and then subsequently amplified using primers complementary to each ligated oligonucleotide (Fig.1, Fig.2A-D; col.2, lines 20-22, 37-45, 52-56; col.3, lines 56-67; col.5, lines 17-19 and Example 1). Uematsu states that the inventive method relies on the primers being of different nucleotide sequences, but of the same length and the same melting temperature. Such primers are composed of the same modules of nucleotide

sequences, but of different orders, and therefore, the nucleotide composition of each primer in its entirety is never changed. For example, one primer can be of the order A-B-C-D-E and another primer can be of the order C-D-A-B-E, where A,B,C,D,E are each composed of 4 nucleotides (col.2, lines 42-45; col.3, lines 16-52; col.4, lines 56-59; col.5, lines 13-15).

Uematsu does not discuss the method of claim 1 wherein a gene-specific primer is also used with the source-specific primer during PCR (i.e. step (b)). Uematsu also does not discuss the method wherein the sequences are detected with bioluminescence analysis, particularly one based on the quantitative determination of pyrophosphate released from an extension reaction [claim 7].

Kato discusses a similar method where isolated mRNA from different sources are converted to cDNA, ligated with a different adaptor for each cDNA, mixed in equal amounts, and amplified using an adaptor primer and a gene-specific primer (see abstract, Fig.1; col.2; col.3, lines 8-11; col.4, lines 23-33, 48-55; col.5, lines 3-5). Kato differs from the instant claimed invention in that he specifically discusses that the adaptor primer (i.e. the source-specific primer) is universal primer, which hybridizes to both selective adaptors.

Although Kato describes autosequencing the amplified product (col.5, lines 3-7), he doesn't specifically discuss performing bioluminescence analysis for sequencing.

Kaufman discusses a method of cataloging complex nucleic acid samples, by ligating sequences called Binary Encoded Sequence Tags (BEST). The method can be used to incorporate sequence information into the nucleic acid, serving as fingerprints of

the nucleic acid samples which can be used for both detection of related nucleic acid samples comparison of nucleic acid samples (i.e. various organisms, etc.), and detection of changes and differences in gene expression patterns of mRNA from different cell samples (col.5, lines 15-35). In one embodiment, Kaufman discusses using the method and detecting the sequences via pyrosequencing (i.e. bioluminescence analysis). The method comprises making double standed cDNA, digesting the cDNA, ligating different adaptors to each sample, amplifying using primers directed to the adaptors, and subjecting the amplified DNA fragments to pyrosequencing (col.51-53).

Regarding claim 8, Kaufman discusses the pyrosequencing using a sequencing primer complementary to one of the adaptor sequences, wherein the unknown adjacent bases can be directly determined via standard pyrosequencing (col.53, lines 4-14).

It is noted that Kaufman doesn't particularly discuss the extension reaction involving the quantitative determination of pyrophosphate released involving a polymerization reaction when deoxynucleotides (dNTPs) are added in a given order, or dideoxynucleotides (ddNTPs) are added in a given order, or an analog of dNTP or ddNTP added in a given order is complementary to the template, although, it is well known in the art that standard pyrosequencing involves the addition of such nucleotides in an iterative fashion as evidenced by Ronaghi et al. Therefore, one of skill in the art can assume that the pyrosequencing reaction of Kaufman et al. also involved such steps and components.

Regarding claim 9, Kaufman also discusses the method of claim 8 wherein the single-stranded PCR products are obtained by treating the PCR products of claim 1 with a physical method or a chemical method, the physical method being to use a biotinylated primer for PCR amplification and then to prepare single-stranded DNAs by a solid phase method, and the chemical method being to use an enzyme for the digestion to prepare single-stranded DNAs. Specifically, Kaufman discusses using a biotinylated PCR primer, such that single-stranded amplicons are retained for further steps (i.e. prepared by solid phase) (col.53, lines 1-3).

One of ordinary skill in the art would have been motivated to modify the method of Uematsu et al. to use gene-specific primers along with the source-specific primers because Kato demonstrates that it was conventional in the art at the time of the invention to conduct a reaction involving ligation of selective adaptors to fragmented DNA, and subsequently amplifying the ligated fragments using a gene-specific primer along with a primer specific for the ligated adaptors, and states that the method resolves the complexity of conventional PCR which requires the preparation of a calibration curve for each of the target genes, making it easy to quantitatively determine gene expression for multiple samples (col.1, lines 25-40). One of ordinary skill in the art would have been motivated to modify the method of Uematsu et al. to detect the amplified sequences with bioluminescence analysis because the benefits of using pyrosequencing (i.e. bioluminescence analysis) following a similar PCR reaction using primers specific for ligated adaptors on the DNA sequence of interest was shown by Kaufman et al. Furthermore, Ronaghi et al. provides evidence that pyrosequencing was

a conventional method in the art for sequence analysis at the time of the invention. Therefore, the skilled artisan would have had a reasonable expectation of success in using a gene-specific primer along with the source-specific primers, as well as detecting via bioluminescence analysis in the method of Uematsu et al. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed gene-specific primer and bioluminescence analysis therein.

7. Claims 1-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uematsu et al. (US 6,225,064) in view of Kwon et al., "Efficient amplification of multiple transposon-flanking sequences," J. Microbiol. Methods, 2000, Vol.41, pp.195-199 and Kaufman et al. (US 6,383,754) as evidenced by Ronaghi et al., "A Sequencing Method Based on Real-Time pyrophosphate," Science, 1998, Vol.281, pp.363 and 365 (of record).

The teachings of Uematsu et al. are discussed above. Uematsu does not discuss the method of claim 1 wherein a gene-specific primer is also used with the source-specific primer during PCR (i.e. step (b)). Uematsu also does not discuss the method wherein the sequences are detected with bioluminescence analysis, particularly one based on the quantitative determination of pyrophosphate released from an extension reaction [claim 7]. Uematsu also does not discuss the method of claim 4, wherein the adaptor is double stranded DNA which contains a part of sequences complementary to recognition sequences of the restriction endonuclease and can be

fully ligated with restriction enzyme cutting ends in DNA fragment by a DNA ligase, a 5' terminal region of one of the strands in the adapter containing a sequence specific to gene sources, a 3' terminal region of the other strand in the adapter containing bases non-complementary to a opposite strand, or a 3' end of the other strand in the adapter being modified to block ability of extension reaction by DNA polymerase, and the adapter having a structure of a "Y" shape consisting of two strands, one end of the adapter being divided into two branches due to no complementary bases, and the other end being formed of a shape of restriction enzyme cutting site [i.e. claim 5]

Kwon et al. discuss a similar method where wild-type and mutant DNA samples are digested, and ligated with a Y linker wherein one end of the linker is divided into two branches due to no complementary bases, and the other end is formed of a shape of restriction enzyme cutting site, and the Y linker comprises sequences complementary to the sticky end generated by the restriction enzyme used in digestion, a 5' region on one of the strands containing a sequence specific for the Y linker-specific primer (i.e. sequences specific to gene sources), and a 3' terminal end of the other strand containing sequences non-complementary to a opposite strand (see abstract Fig.1, pp.197, and pp.198,.1$^{st}$ column). The ligated linker DNA fragment is then subjected to PCR using a primer targeting the transposon (i.e. gene-specific primer) and a Y linker primer (i.e. a source-specific primer). Kwon differs from the instant claimed invention in that he specifically discusses using only one Y-linker primer (i.e. the source-specific primer).

Kwon does not discuss the method wherein the sequences are detected with bioluminescence analysis.

Kaufman discusses a method of cataloging complex nucleic acid samples, by ligating sequences called Binary Encoded Sequence Tags (BEST). The method can be used to incorporate sequence information into the nucleic acid, serving as fingerprints of the nucleic acid samples which can be used for both detection of related nucleic acid samples comparison of nucleic acid samples (i.e. various organisms, etc.), and detection of changes and differences in gene expression patterns of mRNA from different cell samples (col.5, lines 15-35). In one embodiment, Kaufman discusses using the method and detecting the sequences via pyrosequencing (i.e. bioluminescence analysis). The method comprises making double standed cDNA, digesting the cDNA, ligating different adaptors to each sample, amplifying using primers directed to the adaptors, and subjecting the amplified DNA fragments to pyrosequencing (col.51-53).

Regarding claim 8, Kaufman discusses the pyrosequencing using a sequencing primer complementary to one of the adaptor sequences, wherein the unknown adjacent bases can be directly determined via standard pyrosequencing (col.53, lines 4-14).

It is noted that Kaufman doesn't particularly discuss the extension reaction involving the quantitative determination of pyrophosphate released involving a polymerization reaction when deoxynucleotides (dNTPs) are added in a given order, or dideoxynucleotides (ddNTPs) are added in a given order, or an analog of dNTP or ddNTP added in a given order is complementary to the template, although, it is well

known in the art that standard pyrosequencing involves the addition of such nucleotides in an iterative fashion as evidenced by Ronaghi et al. Therefore, one of skill in the art can assume that the pyrosequencing reaction of Kaufman et al. also involved such steps and components.

Regarding claim 9, Kaufman also discusses the method of claim 8 wherein the single-stranded PCR products are obtained by treating the PCR products of claim 1 with a physical method or a chemical method, the physical method being to use a biotinylated primer for PCR amplification and then to prepare single-stranded DNAs by a solid phase method, and the chemical method being to use an enzyme for the digestion to prepare single-stranded DNAs. Specifically, Kaufman discusses using a biotinylated PCR primer, such that single-stranded amplicons are retained for further steps (i.e. prepared by solid phase) (col.53, lines 1-3).

One of ordinary skill in the art would have been motivated to modify the method of Uematsu et al. to use gene-specific primers along with the source-specific primers, and the adaptor being Y shaped as described in claim 5 because Kwon demonstrates that it was conventional in the art at the time of the invention to conduct a reaction involving ligation of selective Y shaped adaptors to fragmented DNA, and subsequently amplifying the ligated fragments using a gene-specific primer along with a primer specific for the ligated adaptors, and states that the method has "general application for the specific amplification of the sequences that flank the known region," and provides a method for analyzing multiple sequences for sequence variations (pp.198, last paragraph). One of ordinary skill in the art would have been motivated to modify the

method of Uematsu et al. to detect the amplified sequences with bioluminescence analysis because the benefits of using pyrosequencing (i.e. bioluminescence analysis) following a similar PCR reaction using primers specific for ligated adaptors on the DNA sequence of interest was shown by Kaufman et al. Furthermore, Ronaghi et al. provides evidence that pyrosequencing was a conventional method in the art for sequence analysis at the time of the invention. Therefore, the skilled artisan would have had a reasonable expectation of success in using a gene-specific primer along with the source-specific primers, adaptors which are Y shaped, as well as detecting via bioluminescence analysis in the method of Uematsu et al. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed gene-specific primer, Y shaped adaptors, and bioluminescence analysis therein.

## ***Summary***

8. No claims are free of the prior art.

9. Nader et al., "Multiplex Pyrosequencing," Nucleic Acids Research, 2002, Vol.30, No.7, pp.1-5; and Weissman et al. (US 6,235,502) are noted as references of interest.

## ***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Molly E. Baughman whose telephone number is 571-272-4434. The examiner can normally be reached on Monday-Friday 8-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Molly E Baughman
Examiner
Art Unit 1637

MEB 9/17/07

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

9/17/07